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PB-288 457

Teratology of Guthion

Midwest Research Inst, Kansas City, MO

Prepared for

Health Effects Research Lab, Research Triangle Park, NC

Aug 78

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

EPA-600/1-78-056
August 1978

Research and Development

PB 288457

EPA

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-78-056		PB288457

4. TITLE AND SUBTITLE	5. REPORT DATE
TERATOLOGY OF GUTHION	August 1978
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Robert D. Short, Jan L. Minor, Timothy M. Unger, and Cheng-Chun Lee	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Midwest Research Institute 425 Volker Blvd. Kansas City, MO 64110	1EA615
	11. CONTRACT/GRANT NO.
	68-02-2746
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory RTP,NC U.S. Environmental Protection Agency Office of Research and Development Research Triangle Park, N.C. 27711	14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The purpose of this study was to assess the effects of Guthion, a pesticide with anticholinesterase activity, on development in rats and mice. A preliminary toxicity study with Guthion indicated that a 35 LD_{50} dose for virgin rats and a 10 day LD_{50} dose for virgin mice was between 4 and 8 mg/kg/day for both species. On the basis of this data, doses of 0, 1.25, 2.5, and 5.0 mg/kg/day were selected for the developmental study, which consisted of two phases. During the first phase, pregnant rats and mice were treated for 10 days starting on gestational day 6. The high dose affected maternal welfare only in rats. Guthion did not significantly increase in a dose-related manner any of the specific anomalies observed in either rats or mice. During the second phase, pregnant rats were treated from gestational day 6 to post-partum day 21. Dams in the high dose group were more sensitive to Guthion later in gestation with the result that deaths and signs of anticholinesterase toxicity increased during this time. Guthion also adversely affected maternal welfare in this group. As a result of Guthion toxicity, only one litter survived until weaning. The inability to dissociate toxicity in adult and developing animals suggests that Guthion has little primary effect on the development of rats or mice.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
pesticides cholinesterase inhibitors	Guthion teratology	06 T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	29
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	A03-A01

EPA Form 2220-1 (9-73)

EPA-600/1-78-056
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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The study described in this report was conducted under our program to determine the health implications of substances used as pesticides, in an effort to evaluate the teratogenic potential of Guthion.

F. G. Hueter, Ph. D.
Acting Director,
Health Effects Research Laboratory

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SUMMARY

The purpose of this study was to assess the effects of Guthion, a pesticide with anticholinesterase activity, on development in rats and mice. A preliminary toxicity study with Guthion indicated that a 35 day LD_{50} dose for virgin rats and a 10 day LD_{50} dose for virgin mice was between 4 and 8 mg/kg/day for both species. On the basis of this data, doses of 0, 1.25, 2.5 and 5.0 mg/kg/day were selected for the developmental study, which consisted of two phases. During the first phase, pregnant rats and mice were treated for 10 days starting on gestational day 6. The high dose affected maternal welfare only in rats. Guthion did not significantly increase in a dose-related manner any of the specific anomalies observed in either rats or mice. During the second phase, pregnant rats were treated from gestational day 6 to postpartum day 21. Dams in the high dose group were more sensitive to Guthion later in gestation with the result that deaths and signs of anticholinesterase toxicity increased during this time. Guthion also adversely affected maternal welfare in this group. As a result of Guthion toxicity, only one litter survived until weaning. The inability to dissociate toxicity in adult and developing animals suggests that Guthion has little primary effect on the development of rats or mice.

TERATOLOGY OF GUTHION

I. INTRODUCTION

The purpose of this study was to evaluate the teratogenic potential of Guthion, a pesticide with anticholinesterase activity. The study consisted of two phases. The first phase was a toxicity study and involved treating virgin female rats and mice with Guthion. The purpose of this phase was to establish doses for the second phase. During the second phase, pregnant rats and mice were treated during gestation with Guthion and their fetuses were examined for abnormalities. In addition, some pregnant rats were treated during gestation and lactation and their pups were observed until weaning.

II. METHODS

A. Animals

Charles River CD® rats weighing 200 to 220 g and CD®-1 mice weighing 20 to 22 g (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were housed in our animal quarters for at least 7 days prior to use. The quarters were maintained at 22 to 26°C with a relative humidity of 40 to 60% and a 7:00 AM to 7:00 PM photoperiod. Animals were given free access to tap water and rodent chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illinois).

Pregnant animals for the teratology study were mated in our animal quarters. Females were housed overnight with a male of the same species. The following morning, rats were examined for sperm in vaginal smears and mice were examined for vaginal copulatory plugs. The presence of these signs was taken as evidence of successful mating, which was identified as day 0 of gestation.

B. Guthion and Preparation of Dose

A sample of Guthion (Azinphosmethyl, Lot No. M007, technical grade), which was received from Environmental Protection Agency (Research Triangle Park) on September 22, 1977, was used in this study. The Guthion was administered orally in a vehicle of cold pressed corn oil (Hain Pure Food Co., Los Angeles, California) which was reported to be free of preservatives. This vehicle was selected to prevent a possible interaction between preservatives and Guthion. All doses were administered in a volume of 5 ml/kg of corn oil.

The high dose was prepared by dissolving a weighed amount of Guthion in acetone, adding the calculated volume of corn oil, and heating the solution for 20 min at 65 to 75°C to volatilize the acetone. Lower doses were prepared by serial dilutions of the high dose. The volume of acetone prior to heating was calculated to represent 2% of the final volume of corn oil. The control group received a solution of corn oil and 2% acetone which was heated for 20 min at 65 to 75°C. All solutions were prepared fresh daily and administered at room temperature.

C. Toxicity

Virgin female rats and mice were treated with daily oral doses of 0, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/kg of Guthion. The body weight on the first day of treatment was used for calculating all doses. Rats were treated for 35 days and mice were treated for 10 days. All animals were observed for 10 additional days at the end of the treatment period.

D. Teratology

Pregnant rats and mice were treated with daily oral doses of 0, 1.25, 2.5 and 5.0 mg/kg of Guthion. The body weight on day 6 of gestation was used for calculating all doses. For rats, each group was divided into two sub-groups. The first sub-group was treated from day 6 to 15 of gestation and sacrificed on day 20 of gestation for examination of the fetuses. The second sub-group was treated from day 6 of gestation until the pups were weaned, 21 days after birth. For mice, dams were treated from day 6 to 15 of gestation and sacrificed on day 18 of gestation for examination of the fetuses.

For the fetal examinations, pregnant animals were sacrificed by cervical dislocation and a laparotomy was performed. The number of live, dead and resorbed fetuses was determined. The umbilical cord was clamped and severed distally to prevent blood loss. Live fetuses were removed, weighed, and immediately examined for external anomalies. One-half of the viable fetuses from each litter was fixed in Bouins fluid for 2 weeks. The hardened fetuses were serially sectioned from the head through the trunk into 1-mm thick sections and examined for soft-tissue anomalies.[1/] The remaining fetuses were fixed in 70% alcohol for 2 weeks and eviscerated. The fetuses were stored in 1% KOH for 2 days and then stained with alizarin red.[2/] After differential decolorization, the skeletons were examined for anomalies.

E. Statistics

Data were analyzed for homogeneity by Bartlett's test.[3] Homogeneous data were analyzed by Dunnett's procedure.[3] Heterogeneous data were analyzed by a nonparametric rank test.[4] The level of significance was selected as $p < 0.05$ unless otherwise indicated. The litter was considered the experimental unit of observation. For example, the percent of fetuses with a given anomaly was calculated for each litter and these values were averaged and expressed as the mean $\pm$ standard error (S.E.) for a treatment group. Therefore, the mean value provides a measure of the affected fetuses per litter, and the standard error provides an estimation of the distribution of effect between litters within a group. Data are reported as the mean $\pm$ S.E.

III. RESULTS

A. Toxicity Study

The mortality in rats treated with Guthion for 35 days and mice treated with Guthion for 10 days is presented in Table 1. All rats treated with 8 or 16 mg/kg/day of Guthion died within 5 or 2 days, respectively. Deaths which occurred at lower doses were attributed to injury produced by dosing as evidenced by tearing of the esophagus. Therefore, deaths which were due to Guthion toxicity occurred only in rats treated with 8 or 16 mg/kg/day. Other signs of toxicity included salivation, urination, lacrimation and tremors. These signs were observed after treatment with 8 and 16 mg/kg/day and occurred within an hour of dosing. Rats exhibited these signs of toxicity for several hours and appeared to be asymptomatic the next day. Similar signs of toxicity were not evident during the 35-day treatment period with lower doses. No signs of toxicity were observed in any of the surviving rats during the 10-day recovery period.

In mice, similar effects were observed (Table 1). All deaths, which were attributed to Guthion toxicity, occurred in the groups that received 8 or 16 mg/kg/day. Other signs of toxicity in these groups included salivation, urination, lacrimation and tremors. The duration of these signs in mice was similar to the duration in rats. No signs of toxicity were observed in mice treated with doses of 4.0 mg/kg/day or lower of Guthion or in surviving mice during the 10-day recovery period.

B. Teratology Study in Rats and Mice Treated with Guthion

1. Maternal Welfare and Reproduction

a. Rats: Adverse effects on maternal welfare were observed only in rats that received 5 mg/kg/day of Guthion (Table 2). These effects included reduced weight gain and feed consumption during the treatment period. These changes were reversible since the values returned to normal when treatment was terminated. Signs of anticholinesterase toxicity, which included tremors, salivation, and urination, occurred in a few rats from this group. Although one death was observed in this group, the data are insufficient to evaluate the effect of pregnancy on toxicity. The remaining groups of rats appeared to be normal. In addition, treatment did not significantly affect the litter size, incidence of resorptions, or fetal body weight in any of the groups tested.

b. Mice: Guthion did not affect the maternal weight change or feed consumption at any of the doses tested (Table 3). Mice and rats demonstrated similar signs of anticholinesterase toxicity to 5 mg/kg/day of Guthion; however, the incidence of these signs was lower in mice. Although one death was observed in the high dose group, no conclusions can be made concerning the effect of pregnancy on toxicity. Guthion, in addition, did not alter the litter size, incidence of resorptions, or fetal body weight in any of the groups tested.

2. External Anomalies

a. Rats: Umbilical hernia and hematoma on the left foot were observed in single fetuses from different litters in the group that received 1.25 mg/kg/day of Guthion. Hematoma on the trunk was also observed in a single fetus from the group that received 2.5 mg/kg/day of Guthion. No external anomalies were observed in any of the other groups.

b. Mice: No external anomalies were observed.

3. Soft Tissue Anomalies

a. Introduction: The soft tissue anomalies observed in rats and mice are summarized and ranked in Table 4. All anomalies were assigned a rank which reflects our opinion of their value in predicting teratogenic potential. According to this ranking, anomalies with a rank of 1 are considered to have little value in predicting teratogenic potential. These anomalies may occur spontaneously or represent an artifact of preparation. In contrast, anomalies with a rank of 3 occur infrequently in controls and are indicative of a disruption in normal development which may compromise survival. Anomalies with a rank of 2 are thought to be of intermediate value in predicting teratogenic potential.

b. Rats: These anomalies are summarized and ranked in Table 5. Guthion treatment at doses of 1.25 mg/kg/day was associated with an increase in slight lateral and fourth ventricle hydrocephalus. The incidence of this anomaly was not related to the dose. The summary of anomalies by rank indicated that Guthion did not significantly increase the incidence of anomalies which were felt to be predictive of teratogenic potential.

c. Mice: These anomalies are summarized and ranked in Table 6. Guthion treatment was not associated with a statistically significant increase in any of the reported anomalies. When the anomalies were summarized by rank, there was a significant increase in the combined anomalies with ranks of 2 and 3 in the groups treated with 2.5 and 5.0 mg/kg/day of Guthion. In addition, anomalies with a rank of 3 were observed only in the groups treated with Guthion.

4. Skeletal Anomalies

a. Introduction: The anomalies observed in rats and mice are summarized and ranked in Table 7. The previously described criteria was used in assigning a rank to individual anomalies.

b. Rats: These anomalies are summarized and ranked in Table 8. Guthion did not significantly increase in a dose-related fashion any of the skeletal anomalies observed in rats.

c. Mice: These anomalies are summarized and ranked in Table 9. Guthion significantly increased the incidence of malaligned sternebrae in the group that received 5.0 mg/kg/day. The anomalies with a rank of 2 were also increased in this group.

C. Toxicity of Guthion in Rats Treated During Gestation and Lactation

Guthion produced death, and reduced both the feed consumption and weight gain of dams treated with 5.0 mg/kg/day from gestational day 6 until postpartum day 21 (Table 10). Most of these deaths occurred prior to delivery. In addition, the incidence of dams demonstrating anticholinesterase toxicity increased prior to birth. None of the other doses produced death or reduced the feed consumption and weight gain of dams similarly treated. The duration of gestation ranged from 21 to 23 days.

Pup weight (Table 10) was adversely affected by 5.0 but not 1.25 or 2.5 mg/kg/day of Guthion. The percent of pups suriviving at various intervals was reduced in the group treated with the high dose of Guthion (Table 11). None of the treatments significantly altered the fertility or gestation index (Table 12). However, there was a trend towards a reduced gestation index in the high dose group.

Neuromuscular problems in the offspring were observed in this study. One day after weaning, pups in the single surviving litter of the high dose group were observed to (1) maintain their rear legs at right angles to the body and have muscular incoordination in the use of these legs, (2) have muscle tremors in the tail, and (3) have an upturned snout. In this litter of five pups, these effects were noticeable in two pups and of questionable incidence in two. Similar symptoms were also observed in one pup from the control group. Therefore, attempts to correlate these neuromuscular problems with Guthion treatment were complicated.

Pups were sacrificed at 30 to 40 days of age and preserved in neutral buffered formalin. There were 25 males and 25 females from 13 litters in the control group, 23 males from 11 liters and 20 females from 10 litters in the group treated with 2.5 mg/kg/day of Guthion, and 1 male and 1 female from the group treated with 5.0 mg/kg/day of Guthion. No offspring were saved from the group that received 1.25 mg/kg/day of Guthion.

IV. DISCUSSION

Although an acute oral LD_{50} dose could not be calculated, this value is probably between 4 and 8 mg/kg/day for both mice and rats. The majority of deaths in both species occurred within 5 days from the start of treatment. Other signs of toxicity included salivation, urination, lacrimation and tremors. These symptoms were observed within an hour of dosing with both 8 and 16 mg/kg/day of Guthion and lasted for several hours. Similar signs of toxicity were not observed with lower doses of Guthion in either rats or mice. No signs of delayed toxicity occurred in either species during the treatment or recovery period. On the basis of these results, doses of 0, 1.25, 2.5 and 5.0 mg/kg/day of Guthion were selected for the developmental toxicity studies.

Adverse effects on maternal welfare in rats were observed only in the group that received 5.0 mg/kg/day of Guthion. These effects were a reduced weight gain and feed consumption during the treatment period. Other signs of toxicity in this group included salivation, urination, lacrimation and tremors. In mice, similar parameters of maternal welfare were not significantly affected at any of the doses tested. Mice also responded to 5.0 mg/kg/day of Guthion with salivation, urination, lacrimation and tremors; however, the incidence appeared to be lower. The data from these studies is insufficient to determine if pregnancy affects the toxicity of Guthion.

Guthion, administered for 10 days during gestation, did not affect the litter size, incidence of resorptions, or fetal body weight in either rats or mice. In rats, Guthion did not produce either a dose-related increase in any of the observed anomalies or a characteristic pattern of anomalies.

In mice, none of the individual anomalies increased in a dose-related fashion or produced a characteristic pattern of defects. However, when all of the anomalies were combined by rank there was a significant increase in soft-tissue anomalies with a rank of 2 and 3 in the group that received 2.5 and 5.0 mg/kg/day of Guthion. In addition, skeletal anomalies with a rank of 2 were increased in the group that received 5.0 mg/kg/day of Guthion. Therefore, in mice the combined incidence of anomalies with an intermediate value in predicting teratogenesis was increased; however, there was not a characteristic pattern of defects. This observation suggests that Guthion increased the incidence of naturally occurring anomalies in mice without producing specific defects.

Additional groups of rats were treated with Guthion from gestational day 6 until postpartum day 21. This treatment schedule produced more deaths in dams receiving 5 mg/kg/day of Guthion than the previous 10-day treatment schedule. Most of these deaths occurred prior to delivery and the incidence of rats demonstrating anticholinesterase toxicity also increased during this time. These observations indicated that dams were more sensitive to Guthion later in gestation.

In addition to mortality, treatment during gestation and lactation reduced feed consumption and weight gain of dams treated with 5.0 but not 1.25 or 2.5 mg/kg/day of Guthion. The high dose also reduced the survival and weight gain of pups from these dams. In contrast, neither 1.25 nor 2.5 mg/kg/day of Guthion altered any of the above parameters.

In order to be classified as a teratogen an agent must alter the structure or function of a statistically significant number of young.[5/] An agent is not classified as a teratogen if it only produced fetal death or reduces fetal growth. In addition, an agent is not classified as a teratogen if the dose required to produce an effect in the embryo or fetuses is overtly toxic to the dam. Therefore, the inability to dissociate maternal and fetal effects in the present study suggests that Guthion has little primary effect on development and, according to the above criteria, is not a teratogen in either rats or mice.

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TABLE 1

MORTALITY IN FEMALE RATS AND MICE TREATED WITH GUTHION

Rats

Dose (mg/kg/day)	Actual Number		Deaths on Treatment Day							Corrected Number[b/]	
	Treated	Dead	1	2	3	4	5	13	35	Treated	Dead
0	6	0								6	0
0.5	6	1[a/]						1		5	0
1.0	7	1[a/]				1				6	0
2.0	7	1[a/]				1				6	0
4.0	6	0								6	0
8.0	6	6			3	1	2			6	6
16.0	6	6	1	5						6	6

Mice

Dose (mg/kg/day)	Actual Number		Deaths on Treatment Day							Corrected Number[b/]	
	Treated	Dead	1	2	3	4	5	9	10	Treated	Dead
0	6	0								6	0
0.5	6	0								6	0
1.0	6	0								6	0
2.0	7	1[a/]		1						6	0
4.0	6	0								6	0
8.0	6	6		1	1	3		1		6	6
16.0	6	6	6							6	6

a/ Death attributed to dosing rather than Guthion.
b/ Number of animals that died from dosing were subtracted.

TABLE 2

EFFECT OF GUTHION ADMINISTRATION DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Number Treated	21	21	21	21
Non-pregnant (%)	4(19)	2(10)	1(5)	3(14)
Dead (%)	0(0)	0(0)	0(0)	0(0)
Pregnant (%)	17(81)	19(90)	20(95)	18(86)
Dead (%)	0(0)	0(0)	0(0)	1(6)
Body Weight Change				
During treatment[a]	61 ± 3	57 ± 3	56 ± 3	29 ± 5[d]
After treatment[a]	62 ± 3	56 ± 3	62 ± 2	59 ± 5
Dams corrected[b]	71 ± 4	71 ± 3	67 ± 3	48 ± 5[d]
Feed Intake[c]				
During treatment	25 ± 1	25 ± 1	24 ± 1	19 ± 1[d]
After treatment	28 ± 2	28 ± 1	29 ± 1	29 ± 1
Pregnant Survivors	17	19	20	17
Implants/dam	14.2 ± 0.4	13.4 ± 0.5	13.9 ± 0.4	12.8 ± 0.9
% Viable fetuses	94 ± 2	93 ± 2	94 ± 1	90 ± 4
% Dead fetuses	0	0	0	0
% Early resorptions	5 ± 2	6 ± 2	5 ± 1	6 ± 2
% Late resorptions	1 ± 1	1 ± 1	0	1 ± 1
Dams with complete resorptions	0	0	0	0
Live Litters	17	19	20	17
Fetuses/dam	13.4 ± 0.5	12.5 ± 0.5	13.1 ± 0.4	11.5 ± 1.0
Fetal weight (gm)	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1

a/ Gm/pregnant rat/interval.

b/ Dam body weight (day 20 - day 0) - uterine weight on day 20.

c/ Gm/pregnant rat/day.

d/ Significantly different from control (Dunnett's procedure).

TABLE 3

EFFECT OF GUTHION ADMINISTRATION DURING ORGANOGENESIS ON MATERNAL WELFARE AND REPRODUCTION IN MICE

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Number Treated	23	22	22	22
Non-pregnant (%)	4(17)	4(18)	5(23)	7(32)
Dead (%)	0(0)	0(0)	0(0)	0(0)
Pregnant (%)	19(83)	18(82)	17(77)	15(68)
Dead (%)	0(0)	0(0)	0(0)	1(7)
Body Weight Change				
During treatment[a/]	15.8 ± 0.5	16.5 ± 0.7	16.4 ± 0.5	15.4 ± 0.8
After treatment[a/]	3.5 ± 0.6	3.9 ± 0.5	4.6 ± 0.3	3.6 ± 0.5
Dams corrected[b/]	3.8 ± 0.4	3.9 ± 0.3	3.7 ± 0.4	2.9 ± 0.5
Feed Intake				
During treatment	6.6 ± 0.2	6.6 ± 0.2	6.7 ± 0.1	6.6 ± 0.2
After treatment	5.4 ± 0.5	5.9 ± 0.4	6.4 ± 0.3	5.3 ± 0.4
Pregnant Survivors	19	18	17	14
Implants/dam	9.6 ± 0.3	10.6 ± 0.5	10.1 ± 0.4	9.9 ± 0.5
% Viable fetuses	93 ± 3	93 ± 3	96 ± 2	93 ± 3
% Dead fetuses	0	0	0	0
% Early resorptions	6 ± 2	4 ± 1	2 ± 1	6 ± 3
% Late resorptions	1 ± 1	0	2 ± 1	1 ± 1
Dams with complete resorptions	0	0	0	0
Live Litters	19	18	17	14
Fetuses/dam	8.9 ± 0.4	9.7 ± 0.3	9.7 ± 0.4	9.3 ± 0.6
Fetal weight (gm)	1.37 ± 0.03	1.35 ± 0.02	1.42 ± 0.02	1.37 ± 0.03

a/ Gm/pregnant mouse/interval.

b/ Dam body weight (day 18 - day 6) - uterine weight on day 18.

c/ Gm/pregnant mouse/day.

TABLE 4

SUMMARY OF OBSERVED SOFT TISSUE ANOMALIES BY RANK

Rank 3: Most valuable in assessing teratogenic potential.

Hydrocephalus: Lateral ventricles
Subarachnoidal space enlarged
Displacement of brain
Ectopic heart
Dextrocardia
Ectopic liver
Hemorrhage in live
Small kidney
Ectopic kidney
Duodenum enlarged
Gastroschisis

Rank 2: Intermediate value in assessing teratogenic potential.

Hemorrhage on olfactory bulb
Trachea closed
Hemorrhage in pericardium
Hydronephrosis: Marked
Hydroureter

Rank 3: Least valuable in assessing teratogenic potential

Hydrocephalus: 4th ventricle
Hydrocephalus: Lateral ventricles, slight
Hydrocephalus: 3rd ventricles, slight
Hydrocephalus: 4th ventricles, slight
Nasopharyngeal canal occluded
Nasal passage occluded
Submaxillary gland exposed
Trachea occluded
Stomach distended
Slight enlargement of kidney pelvis
Kidney cortex solidified
Slight enlargement of ureter
Urinary bladder distended
Malplaced testicle

TABLE 5

EFFECT OF GUTHION EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN RATS

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Number of				
Litters inspected	17	19	20	17
Fetuses inspected	109	111	126	92
Soft Tissue Anomalies (Rank)[a]				
Hydrocephalus: lateral ventricles (3)	1.2 ± 1.2[b]	1.1 ± 1.1	0 ± 0	3.9 ± 3.0
lateral ventricles, slight (1)	4.8 ± 1.9	15.9 ± 4.4[c]	11.0 ± 3.6	15.0 ± 6.6
fourth ventricle (1)	1.8 ± 1.3	2.1 ± 2.1	0.8 ± 0.8	3.9 ± 3.0
fourth ventricle, slight (1)	0.8 ± 0.8	6.1 ± 1.9[c]	3.4 ± 1.6	2.0 ± 1.3
third ventricle, slight (1)	0 ± 0	3.1 ± 1.7	0.8 ± 0.8	1.0 ± 1.0
Subarachnoidal space enlarged (3)	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
Displacement of brain (3)	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
Hemorrhage by olfactory bulb (2)	0 ± 0	0.9 ± 0.9	0 ± 0	0 ± 0
Nasopharyngeal canal occluded (1)	2.5 ± 1.4	7.1 ± 2.8	6.9 ± 2.3	1.8 ± 1.3
Nasal passage occluded (1)	4.6 ± 1.8	4.7 ± 2.3	2.5 ± 1.4	2.8 ± 1.5
Trachea closed (2)	0 ± 0	0.9 ± 0.9	1.0 ± 1.0	2.9 ± 2.9
Trachea occluded (1)	10.4 ± 4.0	13.0 ± 3.2	11.2 ± 2.8	17.8 ± 5.9
Ectopic heart (3)	0.8 ± 0.8	0 ± 0	0 ± 0	0 ± 0
Dextrocardia (3)	0 ± 0	0.9 ± 0.9	0 ± 0	0 ± 0
Hemorrhage in pericardium (2)	0 ± 0	0 ± 0	0 ± 0	3.9 ± 3.9
Ectopic liver (3)	0 ± 0	0 ± 0	0.8 ± 0.8	0 ± 0
Hemorrhage in liver (3)	1.7 ± 1.7	0 ± 0	0.8 ± 0.8	0 ± 0
Stomach distended (1)	0 ± 0	0 ± 0	1.7 ± 1.7	0 ± 0
Hydronephrosis: marked (2)	5.9 ± 4.0	2.6 ± 1.8	4.0 ± 1.6	2.7 ± 1.5
slight (1)	3.8 ± 2.1	4.0 ± 1.9	3.0 ± 1.7	0 ± 0
Small kidney (3)	0 ± 0	0 ± 0	0 ± 0	1.0 ± 1.0
Kidney cortex solidified (1)	0 ± 0	0 ± 0	0 ± 0	0.8 ± 0.8
Ectopic kidney (3)	1.7 ± 1.2	2.8 ± 2.0	1.4 ± 1.0	2.8 ± 1.5
Hydroureter: marked (2)	1.0 ± 1.0	2.0 ± 1.4	1.7 ± 1.1	2.5 ± 1.7
slight (1)	0.8 ± 0.8	2.5 ± 1.4	0.8 ± 0.8	1.0 ± 1.0
Urinary bladder distended (1)	8.8 ± 2.9	12.5 ± 4.2	7.7 ± 3.2	4.5 ± 1.7
Malplaced testicle (1)	9.0 ± 2.9	4.6 ± 1.8	9.6 ± 3.8	6.4 ± 2.7
Submaxillary gland exposed (1)	1.0 ± 1.0	0 ± 0	0 ± 0	0 ± 0
Summary by Rank				
1-3	48.1 ± 4.6	61.3 ± 5.7	49.7 ± 5.6	54.6 ± 7.0
2 and 3	11.3 ± 5.1	9.9 ± 3.6	9.6 ± 2.2	16.8 ± 5.4
3	5.4 ± 2.8	4.4 ± 2.2	4.5 ± 1.6	6.7 ± 3.5

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

c/ Significantly different from control ($P < 0.10$, two-sample rank test).

TABLE 6

EFFECT OF GUTHION EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SOFT TISSUE ANOMALIES IN MICE

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Number of				
Litters inspected	19	18	17	14
Fetuses inspected	80	82	80	64
Soft Tissue Anomalies (Rank)				
Hydrocephalus: lateral ventricles, slight (1)	0 ± 0	0 ± 0	0 ± 0	1.8 ± 1.8
Nasal passage occluded (1)	2.2 ± 1.5	0 ± 0	0 ± 0	0 ± 0
Trachea occluded (1)	8.4 ± 3.6	2.5 ± 1.7	2.0 ± 1.3	2.0 ± 2.0
Hemorrhage in pericardium (2)	0 ± 0	1.1 ± 1.1	3.5 ± 2.0	3.0 ± 2.1
Stomach distended (1)	3.4 ± 1.9	1.1 ± 1.1	0 ± 0	2.0 ± 1.4
Duodenum enlarged (3)	0 ± 0	0 ± 0	0 ± 0	1.0 ± 1.0
Hydronephrosis: marked (2)	0 ± 0	1.9 ± 1.9	1.0 ± 1.0	1.0 ± 1.0
slight (1)	2.1 ± 1.4	0 ± 0	0 ± 0	0 ± 0
Small kidney (3)	0 ± 0	0 ± 0	0 ± 0	1.8 ± 1.8
Ectopic kidney (3)	0 ± 0	0 ± 0	1.2 ± 1.2	0 ± 0
Hydroureter (2)	0 ± 0	0 ± 0	1.5 ± 1.5	0 ± 0
Urinary bladder distended (1)	11.9 ± 4.7	6.7 ± 2.8	7.1 ± 3.6	4.6 ± 2.5
Gastroschisis (3)	0 ± 0	1.1 ± 1.1	0 ± 0	0 ± 0
Summary by Rank				
1-3	27.0 ± 5.4	13.2 ± 4.2	15.0 ± 4.2	15.5 ± 5.6
2 and 3	0 ± 0	4.1 ± 2.3	7.2 ± 2.4[c/]	6.8 ± 2.7[c/]
3	0 ± 0	1.1 ± 1.1	1.2 ± 1.2	2.8 ± 2.0

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

c/ Significantly different from control ($P < 0.05$, two-sample rank test).

TABLE 7

SUMMARY OF OBSERVED SKELETAL ANOMALIES BY RANK

Rank 3: Most valuable in assessing teratogenic potential.

Basisphenoid displaced dorsally
Hemi-vertebra

Rank 2: Intermediate value in assessing teratogenic potential.

Parietals incompletely ossified
Squamosal split
Squamosal incompletely ossified
Sternebra lobed
Split sternabrae
Malalignment of fusion of sternabra
Extra ossification between sternabrae
Centri lobed
Split centri

Rank 1: Least valuable in assessing teratogenic potential.

Skull collapsed slight
Skull collapsed marked
Nasal bones unossified
Occipital fontanel enlarged
Interparietal incompletely ossified
Supraoccipital incompletely ossified
Incus unossified
Jugal incompletely ossified
Hyoid bone unossified
Hyoid bone incompletely ossified
Unossification of sternabra
Incomplete ossification of a sternabra
Ribs extra
Incomplete ossification in pelvis
Paws incompletely ossified
Phalanges of paws unossified

TABLE 8

EFFECT OF GUTHION EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SKELETAL ANOMALIES IN RATS

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Number of				
Litters inspected	17	19	20	17
Fetuses inspected	117	123	136	101
Skeletal Anomalies (Rank)[a/]				
Skull collapsed: slight (1)	10.3 ± 3.3[b/]	8.3 ± 3.8	3.1 ± 1.8	11.9 ± 6.3
marked (1)	0 ± 0	1.4 ± 1.0	1.7 ± 1.7	0 ± 0
Nasal bones unossified (1)	0 ± 0	0 ± 0	0 ± 0	1.0 ± 1.0
Occipital fontanel enlarged (1)	1.2 ± 1.2	2.5 ± 1.9	0 ± 0	2.0 ± 2.0
Parietals: incompletely ossified (2)	1.8 ± 1.3	0.7 ± 0.7	2.3 ± 1.3	0.8 ± 0.8
Interparietals: incompletely ossified (1)	1.2 ± 1.2	2.7 ± 1.6	1.0 ± 1.0	1.0 ± 1.0
medially curved (2)	0 ± 0	0 ± 0	1.4 ± 1.4	0 ± 0
Supraoccipital: incompletely ossified (1)	0 ± 0	0.8 ± 0.8	0 ± 0	0 ± 0
Basisphenoid dorsally displaced (3)	0 ± 0	0 ± 0	0 ± 0	3.6 ± 2.5
Squamosal: split (2)	0 ± 0	0 ± 0	0 ± 0	1.6 ± 1.1
incompletely ossified (2)	2.4 ± 2.4	0 ± 0	0 ± 0	0 ± 0
Jugal: incompletely ossified (1)	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
Hyoid bone: unossified (1)	12.9 ± 5.6	7.9 ± 3.9	13.9 ± 6.3	8.8 ± 3.5
incompletely ossified (1)	2.9 ± 1.6	0.7 ± 0.7	0.7 ± 0.7	1.7 ± 1.7
Sternebrae: ossified normally	22.1 ± 6.8	26.3 ± 6.7	27.6 ± 6.3	20.5 ± 5.7
unossified (1)	57.8 ± 9.7	53.9 ± 8.1	48.1 ± 7.8	42.7 ± 7.4
incompletely ossified (1)	41.1 ± 5.5	36.9 ± 4.1	46.6 ± 4.9	36.9 ± 6.2
lobed (2)	0 ± 0	1.3 ± 1.3	0.7 ± 0.7	0 ± 0
split (2)	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
malaligned (2)	0 ± 0	1.6 ± 1.1	2.2 ± 1.2	1.7 ± 1.2
Centra: ossified normally	90.2 ± 3.5	77.8 ± 5.1	80.0 ± 5.5	90.0 ± 3.6
lobed (2)	7.1 ± 2.6	18.9 ± 5.0	16.5 ± 4.5	5.4 ± 3.4
split (2)	3.3 ± 1.5	5.7 ± 1.8	4.9 ± 2.1	1.7 ± 1.2
Vertebrae: Hemi-vertebrae (3)	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
Ribs: extra (1)	12.5 ± 5.7	3.8 ± 1.6	4.1 ± 1.7	9.9 ± 2.9
Pelvis: incompletely ossified (1)	1.2 ± 1.2	1.3 ± 1.1	1.0 ± 1.0	3.9 ± 3.9
Paws: incompletely ossified (1)	5.5 ± 3.2	5.2 ± 4.5	3.0 ± 3.0	2.9 ± 2.9
phalanges unossified (1)	13.0 ± 6.2	16.0 ± 7.0	7.5 ± 5.5	9.9 ± 6.1
Summary by Rank				
1-3	86.3 ± 5.0	94.3 ± 4.6	77.2 ± 5.8	86.4 ± 4.2
2 and 3	13.9 ± 3.9	23.5 ± 5.0	25.2 ± 5.1	17.8 ± 4.3
3	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

TABLE 9

EFFECT OF GUTHION EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF SKELETAL ANOMALIES IN MICE

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Number of				
Litters inspected	19	18	17	14
Fetuses inspected	87	93	85	67
Skeletal Anomalies (Rank)[a/]				
Skull collapsed: slight (1)	3.4 ± 1.9	3.6 ± 2.0	3.3 ± 2.5	4.8 ± 3.7
Interparietal: incompletely ossified (1)	0 ± 0	1.1 ± 1.1	0 ± 0	0 ± 0
medially curved (2)	0 ± 0	0 ± 0	0 ± 0	1.4 ± 1.4
Supraoccipital: incompletely ossified (1)	5.2 ± 2.9	10.1 ± 4.2	7.8 ± 4.6	4.8 ± 3.7
Incus: unossified (1)	1.1 ± 1.1	1.1 ± 1.1	0 ± 0	0 ± 0
Hyoid bone: unossified (1)	5.2 ± 2.9	1.1 ± 1.1	8.2 ± 4.0	0 ± 0
Sternebrae: ossified normally	75.5 ± 6.3	74.6 ± 5.7	70.8 ± 6.1	65.8 ± 5.5
unossified (1)	0 ± 0	0 ± 0	0 ± 0	1.4 ± 1.4
incompletely ossified (1)	19.4 ± 5.5	9.5 ± 2.7	7.3 ± 3.3	8.7 ± 2.8
lobed (2)	0 ± 0	2.0 ± 1.4	2.0 ± 2.0	2.6 ± 1.8
malaligned (2)	6.4 ± 2.2	12.5 ± 4.4	19.0 ± 6.3	24.3 ± 4.8[c/]
extra ossification between (2)	0 ± 0	1.1 ± 1.1	0 ± 0	0 ± 0
Centra: ossified normally	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Ribs: extra (1)	10.4 ± 4.1	16.9 ± 4.0	11.2 ± 4.5	18.5 ± 4.9
Summary by Rank				
1 and 2	39.3 ± 5.8	42.6 ± 5.2	47.9 ± 7.3	48.3 ± 7.1
2	6.4 ± 2.2	15.6 ± 4.7	21.4 ± 6.3	26.9 ± 4.9[c/]

a/ Ranked by increasing value in predicting teratogenic potential.

b/ Mean ± S.E. of the percent of fetuses with the indicated anomaly calculated on a per litter basis.

c/ Significantly different from control ($P < 0.05$, two-sample rank test).

TABLE 10

EFFECT OF GUTHION ADMINISTERED DURING GESTATION AND LACTATION ON MATERNAL WELFARE AND REPRODUCTION IN RATS

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Rats Treated	14	14	14	15
Non-pregnant (%)	1(7)	1(7)	2(14)	2(13)
Dead (%)	0(0)	0(0)	0(0)	2(100)
Pregnant (%)	13(93)	13(93)	12(86)	13(87)
Dead - total (%)	0(0)	0(0)	0(0)	8(62)
Days 6-16	0	0	0	3
Day 16 - delivery	0	0	0	4
Postpartum	0	0	0	1
Feed Intake (gm/rat/day)				
Gestational days 6-20	27 ± 1	26 ± 1	28 ± 1	20 ± 1[a/]
Postpartum days 7-21	68 ± 4	64 ± 5	63 ± 6	47
Body Weight (gm/rat)				
Gestational				
Day 0	241 ± 4	241 ± 4	248 ± 4	239 ± 4
Day 6	275 ± 4	276 ± 5	286 ± 5	276 ± 5
Day 16	331 ± 7	333 ± 7	345 ± 4	293 ± 8[a/]
Day 20	387 ± 9	382 ± 11	400 ± 10	327 ± 13[a/]
Postpartum				
Day 0	311 ± 9	297 ± 7	315 ± 6	260 ± 11[a/]
Day 4	304 ± 8	304 ± 7	320 ± 5	257 ± 14[a/]
Day 7	317 ± 8	316 ± 7	332 ± 4	278 ± 21
Day 14	346 ± 8	344 ± 7	363 ± 4	340
Day 21	342 ± 10	341 ± 6	360 ± 5	346
Duration of Gestation (days)	21.6 ± 0.1	21.8 ± 0.1	21.9 ± 0.1	22.0 ± 0.2

a/ Significantly different from control (Dunnett's procedure).

TABLE 11

EFFECT OF GUTHION ADMINISTERED TO DAMS DURING GESTATION AND LACTATION ON PUP WELFARE

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Viable Litters[a/]				
Birth	13	13	12	6
Day 4	13	13	11	3
Day 7	13	13	11	1
Day 14	13	13	11	1
Day 21	13	13	11	1
Total Implants/Dam[b/]	12.5 ± 1.1	13.8 ± 0.6	13.9 ± 1.1	13.4 ± 0.9
Pups Delivered/Dam	11.8 ± 1.1	11.9 ± 0.8	12.3 ± 1.3	11.2 ± 0.8
Live Pups/Dam				
Birth	11.7 ± 1.1	11.8 ± 0.8	12.2 ± 1.2	10.7 ± 1.1
Day 4	11.6 ± 1.0	10.2 ± 1.3	11.6 ± 1.5	11.7 ± 0.3
Day 7	11.2 ± 1.0	10.0 ± 1.3	11.5 ± 1.5	10.0
Day 14	11.0 ± 0.9	9.9 ± 1.3	11.4 ± 1.4	5.0
Day 21	11.0 ± 0.9	9.9 ± 1.3	11.4 ± 1.4	5.0
Pup Weight (gm/pup)				
Birth	7.1 ± 0.5	6.4 ± 0.3	6.5 ± 0.2	5.7 ± 0.2[c/]
Day 4	9.0 ± 0.4	8.8 ± 0.6	8.6 ± 0.4	5.4 ± 0.5[c/]
Day 7	12.0 ± 0.7	12.5 ± 0.8	12.2 ± 0.6	7.8
Day 14	24.5 ± 1.1	23.7 ± 1.2	22.8 ± 1.1	14.4
Day 21	37.0 ± 1.6	37.0 ± 1.8	34.4 ± 1.6	24.4

a/ Number of litters with at least one viable pup.
b/ Determined by uterine scarring on postpartum day 21.
c/ Significantly different from control (two-sample rank test).

TABLE 12

REPRODUCTIVE INDEXES OF FEMALE RATS GIVEN GUTHION DURING GESTATION AND LACTATION

	Guthion (mg/kg/day)			
	0	1.25	2.5	5.0
Fertility[a/]	93 (66-100)	93 (66-100)	86 (57-98)	87 (59-98)
Gestation[b/]	100 (75-100)	100 (75-100)	100 (73-100)	46 (19-75)
Pup Survival[c/]				
Day 0-4	100 ± 0	86 ± 9	87 ± 9	46 ± 26[d/]
Day 0-7	97 ± 1	85 ± 9	86 ± 9	14 ± 14[d/]
Day 7-14	98 ± 1	100 ± 1	99 ± 1	50
Day 4-21	96 ± 2	95 ± 4	98 ± 1	14 ± 14[d/]

a/ Confirmed pregnancies/sperm-positive females x 100 (95% confidence limits).
b/ Confirmed pregnancies with viable pups/confirmed pregnancies x 100 (95% confidence limits).
c/ Percent of pups surviving during the indicated interval.
d/ Significantly different from control (two-sample rank test).